known as the hysteresis. Using an optical technique, we have examined the hysteresis for two lipids, SOPE (1-Stearoyl-2-Oleoyl-sn-Glycero-3-Phosphoethanolamine) and DSPE (1,2-Distearoyl-sn-Glycero-3-Phosphoethanolamine. SOPE contains a saturated tail and an unsaturated tail, while in DSPE both tails are saturated. We find that the hysteresis exhibits a power law dependence on the temperature ramping rate and that the hysteresis is markedly reduced for the completely saturated lipid DSPE as compared to the mono-unsaturated lipid SOPE. In turn, the hysteresis of SOPE is markedly reduced compared to that of DOPE, a lipid with two mono-unsaturated tails.

3135-Pos Board B182
Preferential Interaction of α-tocopherol with PUFA-containing Lipids Characterized by Isothermal Titration Calorimetry
Justin A. Williams[1], Daniel S. LoCascio[1], Alekos Tsamaloukas[2], Heiko Heerklotz[2], William Stillwell[1], Stephen R. Wassall[1].
[1]IUPUI, Indianapolis, IN, USA, [2]University of Toronto, Toronto, ON, Canada.
It is becoming generally accepted that membranes laterally segregate into patches (domains) of different lipid composition to provide the environment necessary for the function of a resident protein. Liquid ordered (l_o) lipid rafts enriched in saturated sphingolipids and cholesterol are the best known example. Much less studied are liquid disordered (l_d) domains rich in polyunsaturated phospholipids and depleted in cholesterol, the antithesis of rafts. They are the focus of our research. We hypothesize that α-tocopherol (vitamin E), a lipid-soluble antioxidant found in low concentration in plasma membranes, has preferential affinity for polyunsaturated fatty acid (PUFA)-containing phospholipids in these l_d non-raft regions. In this manner protection of the lipid species most vulnerable to peroxidation due to their multiple double carbon bonds, would be optimized. To test this hypothesis we utilize isothermal titration calorimetry (ITC) to assay the partitioning of α-tocopherol between large unilamellar vesicles (LUV) and methyl-β-cyclodextrin (cyd), a water-soluble molecule with a hydrophobic cavity that binds small hydrophobic molecules. The approach emulates one that has successfully been applied to measure the binding of cholesterol and the results of preliminary experiments have shown that α-tocopherol can be bound by cyd. Partition coefficients K_X measured for α-tocopherol as a function of phospholipid unsaturation are presented and compared with values measured for cholesterol that, in contrast to our proposal for α-tocopherol, has poor affinity for PUFA.

3136-Pos Board B183
The Effect of *Trans* Unsaturation on Molecular Organization in a Phospholipid Membrane
Smita P. Soni[1], Jennifer Runyan[1], Garrison Birch[1], Jesse A. Ward[2], Stephanie E. Sen[1], Scott E. Feller[2], Stephen R. Wassall[1].
[1]IUPUI, Indianapolis, IN, USA, [2]Wabash College, Crawfordsville, IN, USA.
Despite recognition that the ingestion of *trans* fatty acids (TFA) formed during the partial hydrogenation of vegetable oils may unfavorably affect biochemical function, the impact on the conformation of the molecules into which they incorporate is unknown. We synthesized analogs of 1-elaidoyl-2-stearoylphosphatidylcholine (*t*18:1-18:0PC) and 1-oleoyl-2-stearoylphosphatidylcholine (*c*18:1-18:0PC) with a perdeuterated 18:0 *sn*-2 chain and employed solid state ^{2}H NMR, complemented by computer simulations, to compare molecular organization in a model membrane containing a single ''manmade'' *trans* or ''natural'' *cis* double bond. Moment analysis of the ^{2}H NMR spectra recorded as a function of temperature showed that the chain melting temperature for the *trans* isomer (31.5 °C) is depressed compared to the *cis* isomer (7 °C), reflecting an ability to pack more favorably in the gel state, an interpretation supported by molecular modeling. The calculated intra-molecular van der Waals' attraction between acyl chains is greater for *t*18:1 than *c*18:1 acid because the *trans* chain adopts a $t^-s^-\Delta s^-t^-$ conformation, as opposed to $t^-s^-\Delta s^-g^-$ in a *cis* chain, around the double bond. The average order parameters evaluated for the perdeuterated *sn*-2 chain of *t*18:1-18:0PC and *c*18:1-18:0PC in the liquid crystalline phase coincide within <5%, a result that was reproduced in molecular dynamics (MD) simulations. The values for the average order parameter are 20% below the equivalent saturated PC (18:0-18:0 PC), which is attributed to the increased disorder in the hydrophobic core arising from differences in chain packing. We now have synthesized analogs with a perdeuterated *t*18:1 and *c*18:1 *sn*-1 chain to directly probe the conformational organization of *trans* vs. *cis* chain. (Supported by ACS, PRF 43281-AC7.)

3137-Pos Board B184
Scaffolded Vesicles as a Model Membrane System
Michael Grossutti, Jay Leitch, Jacek Lipkowski.
University of Guelph, Toronto, ON, Canada.
Biological cell membranes are complex systems that consist primarily of a phospholipid bilayer, into which cholesterol, proteins etc. may be integrated. Due to the complexity of biological cell membranes, it is desirable to develop model membrane systems that can be more easily studied. Scaffolded vesicles are an example of such a system. A scaffolded vesicle model membrane system offers a number of advantages with respect to other model systems. By using a porous material as the scaffold, one can achieve an aqueous environment on both sides of the model membrane. This allows for the study of membrane transport processes. The scaffolds porosity may also allow one to more easily integrate transmembrane proteins into the bilayer. Importantly, such a system would remain accessible to both electrochemical and surface analytical techniques. Using FTIR-ATR spectroscopy, the orientation of 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) coated on the porous scaffold (as a 70:30 DMPC:cholesterol bilayer) will be determined. Proteins may then be incorporated into the bilayer of the scaffolded model membrane system for study.

3138-Pos Board B185
Mechanical Effects of Peripherally Binding Proteins on Membrane Tethers
Benjamin R. Capraro[1], Aiwei Tian[1,2], Michael C. Heinrich[1], Tobias Baumgart[1].
[1]Department of Chemistry, University of Pennsylvania, Philadelphia, PA, USA, [2]Department of Chemical and Biomolecular Engineering, University of Pennsylvania, Philadelphia, PA, USA.
Our understanding of cell membrane remodeling by proteins has been informed largely by observations of membrane tubulation by proteins in vitro and in vivo. Structural and spectroscopic studies have revealed some important details of the interactions between these proteins and lipids. However, a quantitative description of membrane curvature sensing and generation by proteins, which would guide assessment of the roles of specific proteins and evaluation of hypothesized mechanisms of action, is currently lacking.
We are studying membrane curvature sensing and generation by purified proteins using a fluorescence microscopy-based biomimetic curvature gradient manipulation system, the properties of which are described by membrane elasticity theory. Using tethers of controllable curvature pulled from giant vesicles, we monitor protein partitioning between vesicle and tether, and the effects of proteins on tether properties. These measurements direct our assessment and development of statistical mechanical models that clarify the parameters responsible for protein sensing and control of membrane curvature. Our quantitative framework is used with varying membrane composition and solution conditions to reveal subtle differences between various proteins in their membrane restructuring and curvature propensities.

3139-Pos Board B186
Modeling Morphogenesis of Outer Segments of Vertebrate Photoreceptor Cells
Kristopher Daly.
Clark University, Worcester, MA, USA.
The vertebrate eye contains two main types of photoreceptor cells: rods and cones. The outer segment of the rod cells are cylindrical in shape and contain 500-1000 pancake-shaped structures stacked on top of each other, ensheathed within a plasma membrane. Each individual pancake can be thought of as an individual sac with an enclosing membrane, a structure known as a vesicle. The shape of these vesicles is very important since misshaping of the vesicles can lead to loss of eyesight. We will discuss how the quasi-equilibrium shapes of these vesicles could be determined by membrane energetics, and will introduce a Metropolis algorithm to obtain thermodynamically stable vesicle shapes.

Interfacial Protein-Lipid Interactions II

3140-Pos Board B187
Pulmonary Surfactant Protein C Reduces the Size of Liquid Ordered Domains in a Ternary Membrane Model System
Florian Baumgart[1], Luï¿½ s Loura[2], Manuel Prieto[2], Jesï¿½ s Pï¿½ rez Gil[1].
[1]Dep. de Bioquímica y Biología Molecular I, Universidad Complutense, Madrid, Spain, [2]Centro de Química-Física Molecular, Complexo Interdisciplinar, IST, Lisbon, Portugal.
Surfactant protein C (SP-C) is the smallest pulmonary surfactant protein and is required for the formation and stability of surface-active films at the air-liquid interface in the lung. The protein consists of a hydrophobic transmembrane α-helix and a cationic N-terminal segment, which contains two palmitoylated cysteines. In the present work, we compared the effect of native palmitoylated and recombinant non-palmitoylated versions of full length SP-C on the liquid ordered (lo)/liquid disordered (ld) phase coexistence in a ternary membrane model system consisting of DPPC, DOPC and cholesterol. This model has

been previously shown to simulate the phase properties of the lipid components in lung surfactant bilayers and monolayers. Presence of native palmitoylated SP-C reduced the size of lo domains in the DPPC/DOPC/cholesterol membrane model as detected by Förster Resonance Energy Transfer (FRET). Interestingly, very similar effects on the lo/ld equilibrium could be observed in the presence of a recombinant variant of SP-C, in which the two palmitoylcysteines of the native protein had been replaced by phenylalanines. It has been suggested that phenylalanines can act as functional mimics of palmitoylated cysteines in SP-C from some animal species. We therefore propose that the effects of SP-C on domain size could be related to selective interactions of this protein with liquid-ordered membrane regions and that this could be important for SP-C-promoted stabilization of lung surfactant films *in vivo*.

3141-Pos Board B188

Molecular Dynamics Simulations of Model Lung Surfactant Monolayers and Surfactant Protein B Fragment

Santosh Gupta[1], John Bartlett[1], Jennifer Rendell[1], Alan Waring[2], Valerie Booth[1].
[1]Memorial University, St John's, NL, Canada, [2]University of California, Los Angeles, Department of Medicine, CA, USA.

Surfactant Protein B (SP-B) plays an essential role in the proper functioning of lung surfactant. However, the details of how SP-B interacts with lung surfactant lipids to support lung function are poorly understood. The interactions between an SP-B based peptide and lung surfactant lipid monolayers are investigated using molecular dynamics simulations. Mini-B, a peptide of 34 amino acid residues consisting of the N-terminal and C-terminal alpha helices of full-length SP-B, achieves a similar level of function to full length SP-B in rodent models of respiratory distress. The monolayers probed included pure DPPC, pure POPG, and a mix of 7:3 DPPC:POPG. First the most stable configuration of the peptide-monolayer system is sought by allowing the system to evolve through time from different starting configurations and peptide orientations. It is then determined what effect the counter-ion concentration has on the screening of the electrostatic interaction between the negatively charged headgroups of POPG and the positively charged residues of the peptide. Finally, by placing a bilayer of lipids adjacent to the monolayer, in resemblance to the coexisting lipid reservoirs, the influence Mini-B has on the interaction between the monolayer and bilayer is demonstrated.

3142-Pos Board B189

Interactions of SP-B Based Peptide with Lipid and Protein Components of Lung Surfactant

Muzaddid Sarker[1], Alan J. Waring[2], Frans J. Walther[3], Kevin M.W. Keough[1], Valerie Booth[1].
[1]Memorial University of Newfoundland, St. John's, NL, Canada, [2]Department of Medicine, UCLA School of Medicine, Los Angeles, CA, USA, [3]LA BioMed at Harbor-UCLA Medical Center, Los Angeles, CA, USA.

Lung surfactant (LS) is a mixture of lipids and proteins that reduces the surface tension at the alveolar air-water interface and thus prevents lung collapse and enables normal breathing. Surfactant Protein B (SP-B) is an essential component of LS and is absolutely necessary for survival. SP-B is thought to function by facilitating large-scale rearrangements of lipids and stabilizing the structures at various stages of the breathing cycle. However, neither the structural basis for this ability nor the physiological ramifications of lipid rearrangements are yet understood, in part because a high-resolution structure of SP-B is not yet available. Mini-B is a peptide fragment of SP-B that has been shown in *in vitro* and *in vivo* studies to retain similar activity to the full-length protein. Previously, we determined the structure of Mini-B, first in organic solvent hexafluoroisopropanol (HFIP) and then in detergent micelles composed of sodiumdodecylsulfate (SDS) using solution NMR. In our present work, we have studied the interactions of Mini-B with dodecylphosphocholine (DPC) and SDS micelles. DPC and SDS micelles provide an interfacial environment, with lipid headgroups corresponding to the headgroups of the most abundant lipids in LS, phosphatidylcholine (PC) and phosphatidylglycerol (PG) and thus solution NMR studies of interactions between Mini-B and these micelles can provide insight into LS protein-lipid interactions. We have also investigated the interactions of Mini-B with the most abundant surfactant protein SP-A under similar conditions. These studies further the understanding of the mechanisms of SP-B interactions with other surfactant components in native lung environment.

3143-Pos Board B190

Characterization of Transmembrane Peptide-Anchored Lactoferricin in Mixed Lipids

Rachel E. Ellis, Denise Greathouse.
University of Arkansas, Fayetteville, AR, USA.

To investigate the effects of a cationic juxtamembrane sequence on a hydrophobic transmembrane domain, model peptides have been designed with the lactoferricin sequence RRWQWR (LfB) anchored to an α-helical transmembrane peptide (RRWQWR-(spacer)-$(LA)_7$KKA). Spacers to date have included the helix-breaking -GPGG- and the helix-continuing -AA- sequences. The transmembrane domain contains a hydrophobic $(Leu\text{-}Ala)_7$ helical sequence that spans the membrane with two lysine anchors at the C-terminus. Selected alanines, deuterated on the C_α and C_β carbons, were incorporated in the sequences and used for solid-state NMR spectroscopy. Circular dichroism spectra reveal that the presence of the -AA- spacer correlates with a higher helix content in both DMPC and DMPC:DMPG (3:1) bilayer membranes. Solid state ^2H NMR spectra of macroscopically aligned lipid:peptide samples on glass plates reveal in all cases signals from the $C_\beta D_3$ groups and in some cases signals from the individual C_α deuterons. For example, in DMPC and DMPC:DMPG, the $C_\beta D_3$ groups for the peptide with the -GGPG-spacer give very similar quadrupolar splittings, in the range of 7-10 kHz. Interestingly, in DMPC the C_α deuterons, which are often not observed in similar transmembrane peptides, can also be seen for both labeled alanines, at 48 kHz and 70 kHz. In DMPC:DMPG a single $C_\alpha D$ quadrupolar splitting is resolved at 65 kHz. The presence or absence of selected $C_\alpha D$ resonances in NMR spectra of RRWQWR-(spacer)-$(LA)_7$KKA as well as WALP19, each with or without proline, will be considered in oriented samples of different bilayer lipid compositions.

3144-Pos Board B191

Interaction of the Protein Retinitis Pigmentosa 2 (RP2) with Langmuir Phospholipid Monolayers

Eric Demers, Nicolas Belley, Sophie Champagne, Christian Salesse.
Unité de recherche en ophtalmologie, Centre de recherche du CHUQ, Pavillon CHUL, Faculté de médecine, Université Laval, Québec, QC, Canada.

A severe form of retinitis pigmentosa is linked to mutations of the 350 residues protein RP2 (retinitis pigmentosa 2). This protein contains a α/β C-terminal domain, a highly hydrophobic β-helix and two acylation sites at the N-terminal. It localizes predominantly to the membrane. However, the parameters responsible for the modulation of RP2 binding to membranes are still largely unknown. The objectives of this research work were to characterize the membrane binding properties of RP2 using Langmuir monolayers. The complete sequence of RP2 was expressed and high purity was achieved. RP2 was injected into the subphase underneath phospholipid monolayers bearing different fatty acyl chains (length and unsaturation) and polar headgroups. RP2 binding was monitored by surface pressure measurements. The injection of RP2 underneath phospholipid monolayers led to an increase in surface pressure which indicates its membrane binding. The surface pressure data demonstrate that the adsorption kinetics of RP2 is independent of pH but is strongly affected by the ionic strength of the subphase as well as by the type of phospholipid fatty acyl chain (length and unsaturation) and headgroup. For example, on the basis of its maximum insertion pressure, RP2 shows a preferential binding onto saturated phospholipid monolayers which is consistent with its postulated localization to rafts. This interaction has been further studied by infrared sprectoscopy. In solution, the amide I band is centered at 1630 cm^{-1}, indicating the presence of the β-helix. In contrast, when injected into the subphase in the absence and presence of a phospholipid monolayer, the amide I band is shifted to longer wavenumbers with components at 1640 and 1655 cm^{-1}. These data thus suggest that RP2 has a preferential orientation in monolayers where the α/β C-terminal domain is oriented towards the monolayer.

3145-Pos Board B192

Influence of the Lipidation Motif on the Partitioning and Association of N-Ras in Model Membrane Subdomains

Katrin Weise[1], Gemma Triola[1,2], Luc Brunsveld[1,2], Herbert Waldmann[1,2], Roland Winter[1].
[1]Dortmund University of Technology, Dortmund, Germany, [2]Max Planck Institute of Molecular Physiology, Dortmund, Germany.

In a combined chemical biological and biophysical approach using time-lapse tapping-mode atomic force microscopy, we studied the partitioning of differently lipidated N-Ras proteins with various membrane-recognition motifs into lipid domains of canonical model raft mixtures. The results provide direct evidence that partitioning of N-Ras occurs preferentially into liquid-disordered lipid domains, independent of the lipid anchor system. N-Ras proteins bearing at least one farnesyl group have a comparable membrane partitioning behavior and show diffusion of the protein into the liquid-disordered/liquid-ordered phase boundary region, thus leading to a decrease of the unfavorable line tension between domains. In addition, except for the monofarnesylated N-Ras, strong intermolecular interactions foster self-association and formation of nanoclusters at the domain boundaries and may serve as an important vehicle